Reference: *Biol. Bull.* **193:** 107–115. (October, 1997)

Chemosensory Activation of an Antennular Grooming Behavior in the Spiny Lobster, *Panulirus argus,* Is Tuned Narrowly to L-Glutamate

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Abstract. Antennular grooming behavior (AGB) is a stereotyped behavior in crustaceans in which the first pair of antennae, the major olfactory organs, are clasped and wiped repetitively by the third maxillipeds, which also serve as feeding appendages. AGB apparently functions to clear away accumulating debris on or between the antennular aesthetases (olfactory sensilla). The purpose of this research was to determine whether AGB can be activated by chemicals commonly found in food odors. Lobsters were presented, via headset or handheld pipette, with 27 chemicals found in their food. One chemical, L-glutamate, evoked very high frequencies of wiping. Most chemicals tested were not stimulatory and only a few were weakly stimulatory (adenosine-5′-monophosphate, glycine, D-glutamate). This is surprising because previous studies have shown that other behaviors (antennular flick, search) can be evoked by a much broader array of chemicals found in food odorants. On the basis of these results, we propose that chemosensory neurons that specifically detect L-Glu activate AGB through a recently described non-olfactory pathway. Furthermore, we propose that the role of L-Glu in evoking AGB is based on its electrostatic properties. Because it has a high probability of electrostatic adherence to the antennular cuticle, L-Glu is a sensitive indicator of fouling by food-associated chemicals and thus an appropriate compound to stimulate antennular grooming.

Received 18 February 1997; accepted 20 June 1997.

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Abbreviations: antennular grooming behavior (AGB), olfactory receptor neurons (ORNs), adenosine-5′-monophosphate (AMP), D- & L-alanine (D- & L-Ala), L-arginine (L-Arg), L-asparagine (L-Asn), L-aspartate (L-Asp), betaine (Bet), L-cysteine (L-Cys), D- & L-glutamate (D- & L-Glu), glycine (Gly), L-histidine (L-His), L-hydroxyproline (L-Hyp), L-isoleucine (L-Ile), L-lactate (L-Lac), L-leucine (L-Leu), L-lysine (L-Lys), L-methionine (L-Met), ammonium chloride (NH_4Cl), N-methyl-D-aspartate (NMDA), L-phenylalanine (L-Phe), L-proline (L-Pro), L-serine (L-Ser), L-succinate (L-Suc), taurine (Tau), L-threonine (L-Thr), L-valine (L-Val), artificial seawater (ASW).

Introduction

Odorant access and removal from receptors are equally important for effective chemical detection. To facilitate these two processes, animals have evolved short- and long-term mechanisms that include elimination of stagnant boundary layers, alteration and internalization of chemostimulants, and grooming of chemosensory organs. These mechanisms maintain the sensitivity of the receptor cell and, in the case of grooming, the structural integrity of the chemosensory organ.

Crustaceans are good models in which to investigate chemoreception because they respond to chemostimulants with highly stereotypical and quantifiable behaviors. Chemical cues regulate many components of crustacean behavior including food recognition (Hazlett, 1968; Carr, 1978; Carr, 1982; Schembri, 1981; Fine-Levy *et al.,* 1988), courtship behavior (Atema and Engstrom, 1971; Dunham, 1978; Atema *et al.,* 1979; Gleeson, 1980), and predator avoidance (Mackie and Grant, 1974).

Electrophysiological recordings of receptor neurons (*e.g.,* Derby and Ache, 1984; Anderson and Ache, 1985) and behavioral studies employing ablation techniques (Derby and Atema, 1982; Fine-Levy *et al.,* 1988; Daniel and Derby, 1991) have shown that antennules are the primary organs for detecting odors. Therefore, mechanisms that enhance odorant access to and removal from the perireceptor environment of the antennules are essential to crustacean behavior. Such mechanisms include antennular flicking (Snow, 1973; Gleeson *et al.,* 1993),

biochemical removal of stimuli (Gleeson *et al.,* 1987; Carr *et al.,* 1989), and antennular grooming (Bauer, 1989).

Antennular flicking, which occurs at frequencies of 0.5 to 2 Hz in *Panulirus argus* (Schmitt and Ache, 1979), is defined as the vertical deflection of the lateral filament to a position nearly contacting the medial filament (Zimmer-Faust *et al.,* 1984). Antennular flicking reduces the stagnant boundary layers of seawater created by the dense tufts of aesthetascs (Snow, 1973; Gleeson *et al.,* 1993) which are the sensilla housing the dendritic processes of hundreds of olfactory receptor neurons (ORNs) (Grünert and Ache, 1988). This removal mechanism uses movements of the antennules to splay apart the aesthetasc hairs, allowing water to rush in and displace the water collected during the previous flick. Flicking is analogous to a vertebrate sniff (Schmitt and Ache, 1979) because sniffing flushes air from the upper nasal cavity, the location of the olfactory epithelium. In *P. argus,* flicking is activated in a dose-dependent manner by a broad range of chemical stimuli detected by the antennules (Daniel and Derby, 1991).

The aesthetascs of *P. argus* contain membrane-bound enzymes and transporters that serve to eliminate specific chemical stimuli including nucleotides and amino acids (Trapido-Rosenthal *et al.,* 1988; Carr *et al.,* 1989). Ectonucleotidases catalyze the dephosphorylation of adenine nucleotides, creating an odorant different from the one that initially entered the receptor environment. The end product of dephosphorylation, adenosine, a molecule for which there is little receptor sensitivity, is internalized by a specific uptake system (Trapido-Rosenthal *et al.,* 1987). In addition to the adenosine uptake system, an uptake system for taurine has been identified (Gleeson *et al.,* 1987) and uptake systems for other amino acids, including L-glutamate, also exist (Trapido-Rosenthal *et al.,* 1988; Carr *et al.,* 1989). Prolonged stimulation of ORNs limits the temporal and spatial resolution of "patchy" olfactory environments (Atema *et al.,* 1989). Quick elimination of odorants by these biochemical removal mechanisms as well as by antennular flicking enhances receptor sensitivity.

The least studied mechanism for facilitating odorant removal and maintaining receptor accessibility is antennular grooming behavior (AGB). Although AGB is common in many crustaceans, very little is known about which stimuli activate it (Bauer, 1989). This behavior consists of an antennular deflection downward, permitting the lateral and medial antennular filaments to be grasped by the third maxillipeds (paired appendages on either side of the mouth) and pulled repeatedly through the setal combs of the maxillipeds. The resulting action facilitates removal of material that has accumulated on or between the aesthetascs (Snow, 1973; Bauer, 1989). Fouling is a recurring problem for crustaceans because both the exoskeleton and specialized structures such as gills and antennules are favorable substrates for microbes and detritus. If this material is not removed, chitinivorous microorganisms can damage the exoskeleton and the presence of other fouling organisms can cause respiratory and sensory impairment. When a shrimp, *Heptacarpus pictus,* was experimentally prevented from grooming the antennules, extensive structural damage of the aesthetascs occurred (Bauer, 1977). Therefore, anything that enhances the level of microbial fouling is detrimental to the structural integrity of the antennule and, presumably, to its functional role as a chemoreceptor organ.

Although fouling of the antennules occurs continually, feeding may result in particularly high rates of accumulation of debris. By providing a nutrient-rich substrate, this debris facilitates microbial colonization. Results of studies of several crustacean species show that AGB can be elicited in response to compounds typically found in food (Snow, 1973; Zimmer-Faust *et al.,* 1984). In this paper, we examine which chemicals found in the food of *P. argus* stimulate the release of AGB. Compounds found in food, including amino acids, nucleotides, organic acids and ammonium (Carr and Derby, 1986) were tested. Results of behavioral assays revealed that only one amino acid, L-glutamate (L-Glu), evoked AGB with great frequency. Even analogues of L-Glu (D-glutamate, L-aspartate, N-methyl-D-aspartate) were not excitatory. On the basis of these results, we propose that chemosensory neurons that specifically detect L-Glu activate AGB through a recently described non-olfactory pathway (Schmidt *et al.,* 1992; Schmidt and Ache, 1996). Furthermore, we propose that L-Glu evokes AGB because the high probability of electrostatic adherence to the antennular cuticle makes it a sensitive indicator of fouling by food-associated chemicals.

Materials and Methods

Source and maintenance of lobsters

Spiny lobsters (55 to 70 mm carapace length) were obtained from the Florida Keys Regional Marine Laboratory in Long Key, Florida, and maintained in separate 80-l aquaria (one lobster/aquarium). Aquaria were lined with crushed coral; filled with aerated, recirculating Instant Ocean (specific gravity, 1.021–1.023); and equipped with gravel-bottom filter systems. Lobsters were fed scallop or shrimp daily *ad libitum* and the uneaten food was removed after 1 h. The light:dark cycle was 12:12 and the ambient temperature was maintained between 25°–27°C. Red light (25 W, ceramic-coated light bulbs) was provided during the dark cycle.

Chemical Stimuli

The following compounds were used as stimuli: adenosine-5′-monophosphate (AMP), D- & L-alanine (D- & L-

Ala), L-arginine (L-Arg), L-asparagine (L-Asn), L-aspartate (L-Asp), betaine (Bet), D- & L-glutamate (D- & L-Glu), glycine (Gly), L-histidine (L-His), L-hydroxyproline (L-Hyp), L-isoleucine (L-Ile), L-lactate (L-Lac), L-leucine (L-Leu), L-lysine (L-Lys), L-methionine (L-Met), ammonium chloride (NH_4Cl), N-methyl-D-aspartate (NMDA), L-phenylalanine (L-Phe), L-proline (L-Pro), L-serine (L-Ser), L-succinate (L-Suc), taurine (Tau), L-threonine (L-Thr) and L-valine (L-Val). Stock solutions (10 m*M*, pH 8.1) of all the stimuli were prepared in artificial seawater (ASW) (Cavanaugh, 1964). L-cysteine (L-Cys) was prepared on the day of testing to prevent precipitation from freezing; all others were stored at −70°C until used. With the exception of D-Ala, D-Glu (a stereoisomer of Glu), NH_4Cl, and NMDA (an agonist of a subclass of glutamate receptors (Schoepp, 1994)), these compounds were identified by Carr and Derby (1986) as components of prey extracts. On the day of testing, appropriate stock solutions were thawed and diluted to either 5 m*M* or 0.5 m*M* with ASW.

Experimental design

The 26 compounds listed above were each assayed at 0.5 m*M*. To ensure that the lobsters would not become desensitized, we presented these chemicals in five separate trials (maximum of 10 chemicals per trial including ASW & L-Glu) so that no experiment lasted longer than 4 h. We subsequently tested 10 of these compounds (in three separate trials) at 5.0 m*M*. In previous trials, these 10 chemicals had elicited either a highly significant response (*i.e.*, one that was significantly greater than the response toward ASW) or the response was greater than the response toward ASW for a majority of lobsters. The exception was NMDA, which was assayed at both concentrations even though it failed to meet the criteria. Finally, a series of concentrations of L-Glu (0.01, 0.05, 0.08, and 0.1 m*M*) and three other compounds (AMP, Gly, D-Glu at 0.5, 1.0, 5.0 and 10.0 m*M*) shown to elicit significant responses at 5.0 m*M* were tested. Each of the four compounds was assayed in a separate trial. All trials included ASW as a control stimulus and 0.5 m*M* L-Glu, which preliminary experiments showed to be very effective in evoking AGB, as a response standard.

Presentation of stimuli

Experimental trials were blind in that the experimenter did not know the order in which the chemicals were presented. Each stimulus was administered to six lobsters, except where noted in Results, in triplicate tests. Two methods of stimulus presentation were employed: automated and handheld pipette. For the first two trials using stimuli at 0.5 m*M*, presentation of stimuli was automated via a headset apparatus as described previously in Daniel and Derby (1988). A 13-mm diameter acrylic rod was attached by hook and loop fasteners to the rostrum. A bent glass rod (5-mm diameter) was glued to the rostrum attachment with cyanoacrylate quick-bonding nontoxic glue. This provided support for the tubing through which the stimulus was introduced (1-mm inner diameter, attached to the rod with cable ties) and allowed test solutions to be injected at a constant distance in the vicinity of the antennules. A peristaltic pump was used to deliver all solutions. To desensitize the animals to the mechanical stimulus, tank water was pumped continuously, via plastic flexible tubing, from the tank through the stimulus-introduction tubing. Each test solution (5-ml in a 10-ml plastic syringe) was injected into the tubing through a two-way stopcock valve. The flow rate of the pump was maintained at 10-ml · min^{-1}. In subsequent trials, stimuli were presented via a handheld 5-ml pipette. The tip of the pipette was gently placed in the vicinity of the antennules. This method appeared to elicit a consistently greater magnitude of AGB from the lobster (Fig. 1). All trials were videotaped, beginning 0.25 min before each stimulus was presented and continuing for up to 2 min afterward.

Data analysis

The magnitudes of the AGB responses in all experiments were determined from videotapes. Pre-stimulus wipe rates were determined by counting the number of wipes that occurred in the 0.25-min period before stimulus presentation. Post-stimulus wipe rates were determined by recording the number of wipes that occurred for 1 min after stimulus presentation. Wipe rates were therefore defined as the post-stimulus response rate minus the pre-stimulus response rate. The three wipe rates (wipes · min^{-1}) counted for each stimulus per trial were averaged and reported as the mean wipe rate.

In most cases, data did not meet the assumptions necessary for parametric statistical tests; therefore, nonparametric tests were used. Friedman's repeated measures tests on ranks was used to compare responses to chemical stimuli (Sigmastat, Jandel Scientific). Where statistically significant differences were found, pairwise comparisons were performed using the Student-Newman-Keuls (S-N-K) test adapted for ranked data. For the concentration series experiments, least-squares regression analyses were performed on log-transformed concentrations of each stimulus vs. wipe rates standardized to the L-Glu (0.5 m*M*) response.

Results

*Responses to compounds at 0.5 m*M

Of the compounds presented at 0.5 m*M*, L-Glu was by far the most effective compound at eliciting AGB. In trials #1–#4, reported in Figure 1, L-Glu produced significantly

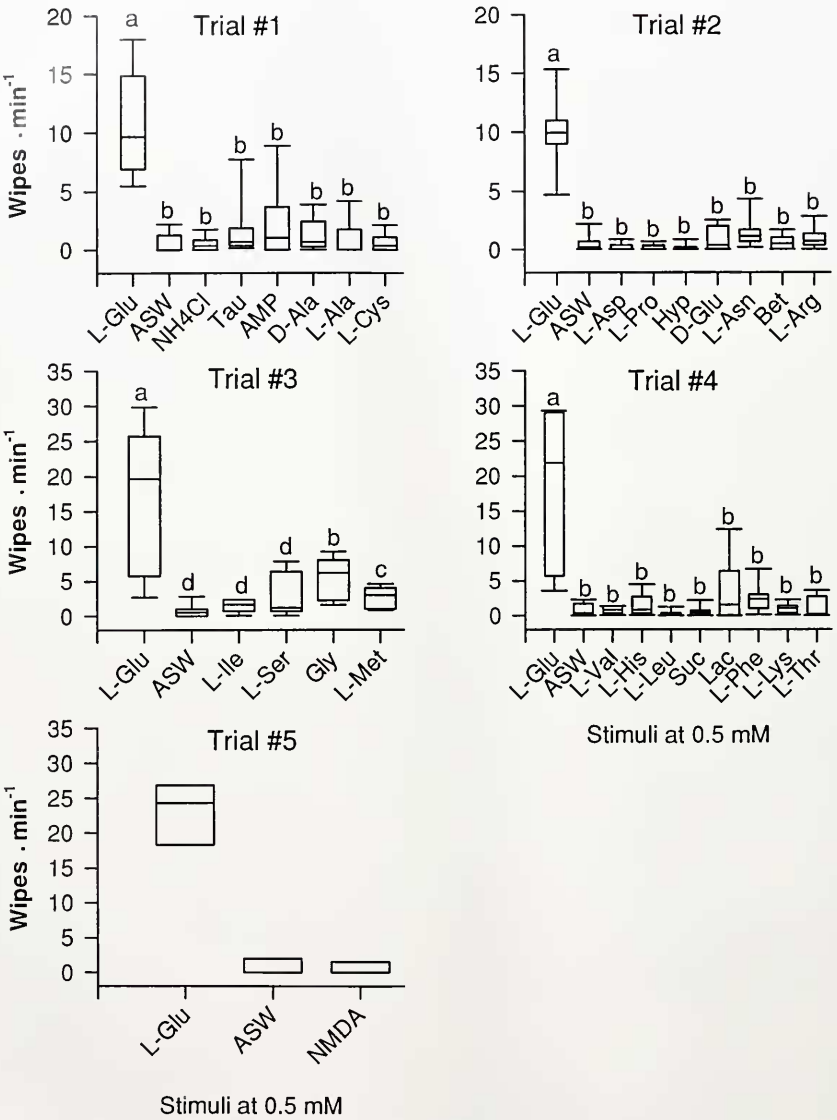

Figure 1. Magnitude of AGB (number of wipes · min^{-1}) towards single stimuli at 0.5 m*M*. Nine lobsters were tested in trials #1 and #2, six in trials #3 and #4, and three in trial #5. Each box plot shows the distribution of lobster responses. The 25th and 75th percentiles are shown as the lower and upper limits of the box and the 50th percentile as the line within the box. The caps on vertical lines show the 10th and 90th percentiles. Stimuli with significantly different wipe rates within a trial are indicated by different letters above box plots. See text for details of statistical results.

higher wipe rates than all other stimuli tested at 0.5 m*M* and ASW (Fig 1; Friedman repeated measures ANOVA; trial #1, $\chi^2 = 29.7$, $n = 9$, $P = 0.0001$; trial #2, $\chi^2 = 34.6$, $n = 9$, $P = 0.0001$; trial #3, $\chi^2 = 24.4$, $n = 6$, $P = 0.0002$; trial #4, $\chi^2 = 23.1$, $n = 6$, $P = 0.006$; S-N-K pairwise comparisons test for ranked data, $P < 0.05$). Two compounds, Gly and L-Met (trial #3), elicited responses that were significantly greater than ASW (S-N-K pairwise comparisons test for ranked data, $P < 0.05$). Neither the stereoisomer of L-Glu, D-Glu, nor the analogue, L-Asp (sidechain has one less carbon), elicited responses greater than those toward ASW in trial #3 (S-N-K pairwise comparisons test for ranked data, $P > 0.05$). In addition, NMDA did not elicit significant responses (trial #5, $\chi^2 = 4.91$, $n = 3$, $P = 0.194$). However, none of the compounds tested in this trial, including L-Glu, were significantly different from ASW. This is probably because the small sample size used in this experiment resulted in a very low-power test. However, all three lobsters tested responded much more to L-Glu than to ASW or NMDA at 0.5 m*M*.

*Responses to compounds at 5 m*M

Responses to L-Glu at 0.5 m*M* were significantly higher than responses to test stimuli when presented at 5.0 m*M* (Fig. 2; Friedman's repeated measures ANOVA; trial #1, $\chi^2 = 43.1$, $n = 9$, $P = 0.0001$; trial #2, $\chi^2 = 24.7$, $n = 6$, $P = 0.0002$; trial #3, $\chi^2 = 10.4$, $n = 6$, $P = 0.0055$; S-N-K pairwise comparisons test for ranked data, $P < 0.05$). Six compounds, L-Asn, L-Cys, AMP, and Tau in trial #1, Gly and D-Glu in trial #2, evoked wipe rates significantly greater than those for ASW (S-N-K pairwise comparisons test for ranked data, $P < 0.05$). NMDA produced no significant response at 5.0 m*M* (trial #3, S-N-K pairwise comparisons test for ranked data, $P > 0.05$).

Responses to concentration series

The magnitude of AGB towards L-Glu increased as a linear function of the log of its concentration (Fig. 3, least-squares regression, $F = 86.6$, $n = 30$, $P < 0.001$, $r^2 = 0.76$, standardized wipes $\cdot$ min^{-1} = 107.7 + (53.1 $\cdot$ log[concentration]) and were at least 100 times more effective than any of the other chemicals across the range of concentrations tested. Only the AMP concentration series yielded a significant regression (least-squares regression, $F = 22.6$, $n = 26$, $P < 0.0001$, $r^2 = 0.55$, standardized wipes $\cdot$ min^{-1} = 7.26 + (39.5 $\cdot$ log[concentration]). From the linear regression equations, the concentrations needed to achieve 25% and 50% maximal responses were 0.03 and 0.08 m*M*, respectively, for L-Glu, and 3.0 and 14 m*M*, respectively, for AMP. Although the linear regression equations for Gly and D-Glu were not significant, the 50% maximal responses based on visual inspection were at least 10 m*M*.

Discussion

AGB specificity to L-glutamate: implications for sensory-motor integration

Our results show that, unlike other behaviors studied in *P. argus* (Fine-Levy *et al.,* 1988, 1989; Daniel and Derby, 1988; Fine-Levy and Derby, 1991, 1992; Lynn *et al.,* 1994), AGB is elicited almost exclusively by one chemical, the L-enantiomer of glutamate (L-Glu). L-Glu was at least 100 times more effective in eliciting AGB than were AMP, Gly, and D-Glu, the next-best single stimuli. Furthermore, the D-enantiomer of L-Glu (D-Glu), a structural analogue of L-Glu (L-Asp), and an agonist of a major class of glutamate receptors (NMDA), either failed to activate AGB (L-Asp, NMDA) or were only weakly effective at high concentrations (D-Glu). We propose that elicitation of AGB requires sensory input from a specific class of chemosensory neurons narrowly tuned to L-Glu.

According to electrophysiological studies, ORNs in *P. argus* are narrowly tuned to specific chemical stimuli and can be classified by best-compound. "Best" cells have been identified for AMP, ATP, Cys, Bet, Glu, NH_4Cl, and Tau (Derby and Ache, 1984; Carr *et al.,* 1986; Derby *et al.,* 1991; Daniel *et al.,* 1994). Similar tuning characteristics have been identified for olfactory and non-olfactory chemosensitive neurons distributed on second antennae, antennules, maxillipeds, and legs of *Homarus americanus* (Johnson *et al.,* 1985; Tierney *et al.,* 1988; Corotto *et al.,* 1992; Voigt *et al.,* 1997). Biochemical receptor-binding assays of antennules of *P. argus* have identified independent olfactory receptor sites for AMP, Tau (Olson *et al.,* 1992; Olson and Derby, 1995; Sung *et al.,* 1996), both stereoisomers of Ala (Michel *et al.,* 1993), and L-Glu (Burgess *et al.,* 1994).

Compounds that weakly evoke AGB may do so by activating L-Glu-best neurons. Electrophysiological studies of ORNs showed that responses to next-best stimuli were generally 100-fold less than to the best compound (Daniel *et al.,* 1994). Hence the amino acids Gly and D-Glu, and the nucleotide, AMP, are possibly "next-best" stimulants that weakly activate AGB via L-Glu-best cells. However, the response spectra of AGB and of ORNs sensitive to L-Glu are not entirely consistent. NMDA and L-Cys serve as partial agonists and antagonists for chemoreceptors presumed to be ORNs sensitive to glutamate (Burgess and Derby, 1995). Since AGB was elicited by L-Glu but not NMDA or L-Cys even at 5 m*M*, it is possible that non-olfactory neurons mediate AGB.

How might such a specific stimulus lead to activation of AGB? There appear to be two antennular chemosensory

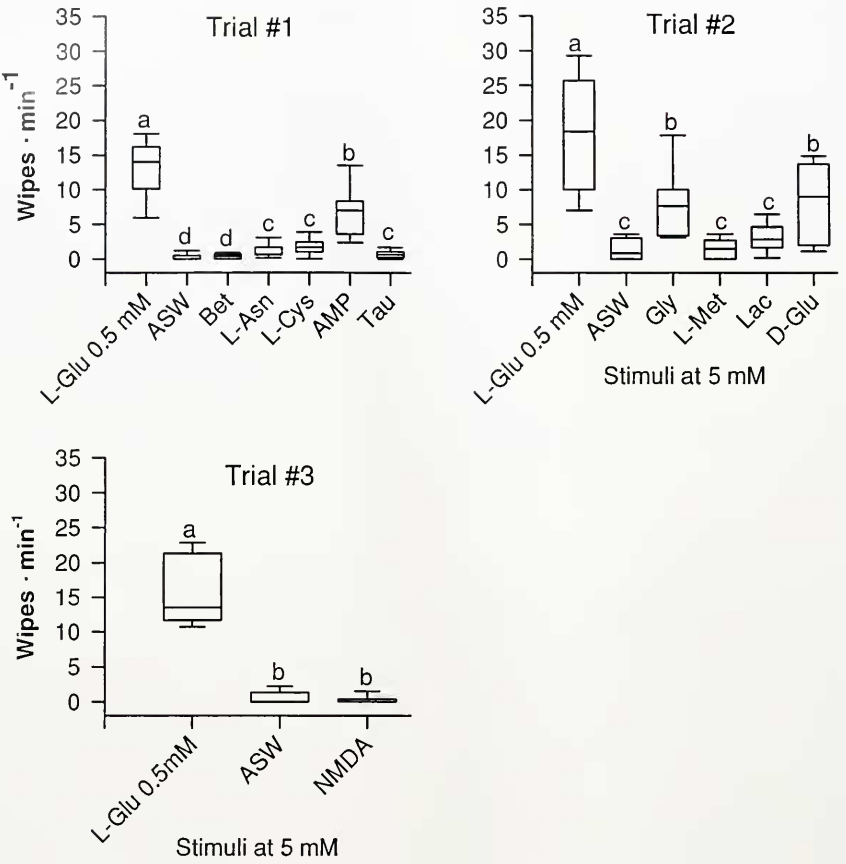

Figure 2. Magnitude of AGB (number of wipes · min $^{-1}$) towards L-Glu at 0.5 m*M* and other single stimuli at 5 m*M*. Nine lobsters were tested in trial #1 and six in trials #2 and #3. Each box plot shows the distribution of lobster responses. The 25th and 75th percentiles are shown as the lower and upper limits of the box and the 50th percentile as the line within the box. The caps on vertical lines show the 10th and 90th percentiles. Stimuli with significantly different wipe rates within a trial are indicated by different letters above box plots. See text for details of statistical results.

processing pathways in the brain of *P. argus*, an olfactory and a non-olfactory pathway. In the olfactory pathway, ORNs with dendrites located within the aesthetases on the distal half of the lateral filament of the antennules project to the olfactory lobe (Schmidt and Ache, 1992). In the non-olfactory pathway, chemosensory neurons (as well as mechanosensory neurons) with dendrites in non-aesthetasc sensilla on lateral and medial filaments of the antennules terminate exclusively in the lateral antennular neuropil (LAN) and the medial antennular neuropil (MAN) in the brain (Schmidt *et al.*, 1992; Schmidt and Ache, 1996). Antennular motorneurons also branch in these neuropils. Similarly, in the blue crab, *Callinectes sapidus*, motorneurons that control such activities as antennular withdrawal and flick originate in these regions (Roye, 1989, 1994).

We propose that the non-olfactory pathway may represent a fairly direct reflexive route for eliciting AGB. According to this hypothesis, non-olfactory chemosensory neurons tuned to L-Glu synapse in the LAN, in the MAN,

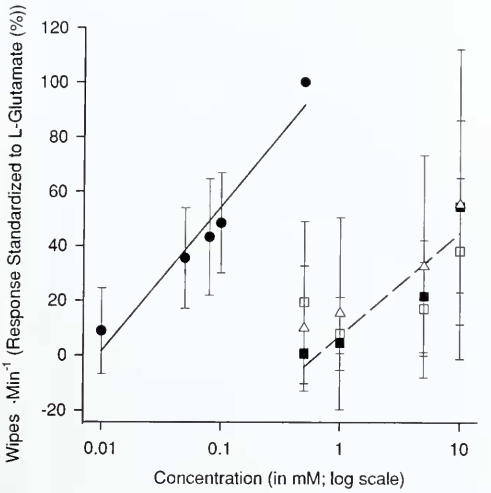

Figure 3. Dose response curves for magnitude of AGB to L-Glu, AMP, Gly, and D-Glu. AGB is expressed as wipes · min^{-1} standardized to the responses to L-Glu at 0.5 m*M*. Dose-response curves fit by linear regression and found to be statistically significant are shown for AMP (dashed) and L-Glu (solid). Symbols (△ = D-Glu, ■ = AMP, ● = L-Glu, □ = Gly) show the means at each log-transformed concentration, and capped bars show ±1 standard deviation unit. See text for details of statistical results.

or in both. We suggest that the motor program for AGB resides in these neuropils. Although the tuning of these chemosensory neurons has not been studied in *P. argus*, in *H. americanus* non-olfactory chemosensory neurons, like ORNs have been shown to be tuned narrowly (Voigt and Atema, 1992). This hypothesis may explain the differences in responses of AGB and of presumed ORNs towards NMDA and L-Cys (Burgess and Derby, 1995) discussed above.

Ablation studies of both lateral and medial filaments performed in our laboratory showed that the antennules are the primary sources of the chemosensory input that evokes AGB (Barbato *et al.*, 1996). Ablation of specific regions of the antennules will provide important evidence as to whether AGB is driven by olfactory or non-olfactory input. AGB in *P. argus* provides a useful model for understanding how specific chemosensory information can drive a fairly complex, stereotyped motor program.

Functional significance of L-glu evoked AGB

If chemosensory-mediated AGB in the spiny lobster is a mechanism for removing antennular debris—as previous studies on other crustaceans suggest (*e.g.*, Bauer, 1977)—it may be that L-Glu is a sensitive indicator of potential biofouling. L-Glu is a common constituent of extracts of typical food (Carr and Derby, 1986; Carr *et al.*, 1996). For example, 5 m*M* complex mixtures mimicking shrimp, mullet, crab, and oyster extracts contain L-Glu concentrations of 0.025, 0.026, 0.034, and 0.06 m*M*, respectively (Carr and Derby, 1986). According to the dose-response curve shown in Figure 3, the predicted AGB response at these concentrations is 23%, 23%, 30%, and 43%, respectively, of the maximum standardized L-Glu response. In fact, we have observed significant levels of AGB elicited by 5 m*M* shrimp and oyster mixtures (Barbato *et al.*, 1996). If lobsters encounter food effluents at these concentrations, which odor plume studies (Moore and Atema, 1991) indicate would probably occur within the immediate vicinity of a food source, there is a good chance that they may be induced by L-Glu to groom their antennules.

In addition, L-Glu is very "sticky" compared to other compounds found in complex mixtures mimicking natural prey extracts. At the pH of seawater, 8.1, L-Glu has three charged functional groups: two negative carboxyl groups, and one positive amino group (Lehninger *et al.*, 1993). Conversely, most of the remaining chemicals found in complex mixtures have a maximum of two charged functional groups. For example, of the 33 chemicals in complex mixtures mimicking oyster extract and the 29 in complex mixtures mimicking shrimp extract (Carr and Derby, 1986), only three amino acids (Asp, Lys, Arg) possess a charge orientation similar to that of L-Glu. This charge orientation may make it possible for an electrostatic attraction to be established between these four amino acids and the cuticle of the aesthetasc. The outer portion of crustacean cuticle is composed of spherulitic calcite islands surrounded by a lipoprotein matrix (Roer and Dillaman, 1984), so the amino acid residues found in the matrix may be the site of the attraction. The four amino acids with the special charge orientation would be more likely to stick to the cuticle surrounding the aesthetasc than other compounds.

Of these compounds, only L-Glu is known to elicit significant excitatory activity from ORNs (Derby and Ache, 1984). Similarly, in our study only L-Glu elicited AGB activity. Therefore, because L-Glu can easily adhere to the cuticle, is commonly found in food, and can be detected by chemoreceptors, it is the most sensitive indicator of potential fouling by food. By keying in on this chemical, the lobster may ensure the complete removal of other fouling material, thus preventing the buildup of debris on the antennules.

Acknowledgments

We thank John Swanson, Bill Gibbs, and the staff of Florida Keys Marine Laboratory in Long Key for provid-

ing lobsters. We thank the three anonymous reviewers for their critique of the manuscript. Research described represents partial fulfillment of requirements for the degree of Master of Arts.

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